Deiodinase Type I, II, and III Expression in Amphibian Pituitary, Thyroid, and Limb Bud at Key Stages of Development and after Exposure to the Thyroid Hormone Synthesis Modulators: Methimazole, Perchlorate and Propylthiouracil

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Objectives

- Determine expression of deiodinases in normal development and after exposure to modulators
- Examine role of deiodinase expression contributing to circulating T_3 in the organism
- Obtain evidence for regulated D1 expression to support authenticity of apparent expression

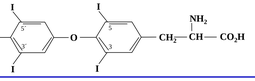
Abstract

Based on recommendations from the Endocrine Disruptor Screening and Testing Program Advisory Committee, the US EPA is developing a screening assay capable of detecting the effects of perturbed thyroid hormone status in Xenopus laevis. One aspect of the assay is developing sensitive molecular measurements to improve diagnostic capability. The major product of the thyroid gland is T_4, however T_3 is known to be the more active form of thyroid hormone (TH). Conversion of T4 to T_3 is catalyzed by type I and II deiodinases (D1 and D2), while type III deiodinase (D3) has a major role in inactivation of T3. Expression measurements of these genes were made by quantitative real-time RT-PCR (Q-PCR) in pituitaries, thyroids, and limbs at key stages of development. Significant patterns of gene expression were observed that are consistent with TH coordinating changes in development. For example, D2 expression peaks in the limb at stage 56, while D3 increases beyond this stage. In the thyroid, D2 peaks much later at stage 63, while D3 still appears to be increasing. These changes are consistent with different tissues requiring different local T3 concentrations at different times. Additionally, the expression of these genes was measured after exposure to methimazole, perchlorate, and propylthiouracil. In the thyroid, D2 expression was increased relative to controls. This is consistent with the organism attempting to produce more of the active form of circulating TH. It has long been thought that D1 does not exist in amphibians, however we were able to show gene expression that appears to be developmentally regulated. Although the presence of a functional D1 activity needs to be confirmed, the temporal expression in select subpopulations of cells within tissues could have important consequences for the timing of developmental changes. This abstract does not necessarily reflect EPA policy.

Deiodinase Properties

$$TI_N + 2RSH \longrightarrow TI_{N-1} + RSSR + I^- + H^+$$

	Main Function	Preferred Substrate	Relative Km	PTU Sensitivity	Subcellular Location
Type I D1 ORD & IRD	TH Degradation Circulating T_3 (?)	rT_3 > T_4, T_3 Sulfation increases	Micromolar	Yes	Plasma Membrane
Type II D2 ORD	Activation	T_4	Nanomolar	No	Endoplasmic Recticulum
Type III D3 IRD	Inactivation	T_3, T_4	Nanomolar	No	Plasma Membrane



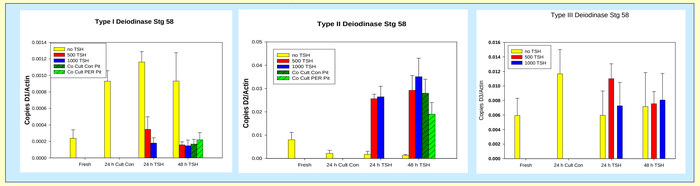
Methods

- RNA was extracted using Qiagen's Micro RNeasy kits and analyzed for quality using an Agilent Bioanalyzer with picochips. RNA concentration was measured on some samples with a Nanodrop spectrophotometer
- Quantitative Real-Time RT-PCR was done on an Applied Biosystems 7500 Real time PCR system using TaqMan EZ RT-PCR kits. Primers and probes were designed with Primer Express software and purchased from IDT technologies. Quantities were determined by absolute quantitation using a standard curve of IVT synthesized RNA.
- Sequence information was obtained from NCBI and TIGR:

D1	BX844453 (TIGR TC251139; see also AAZ430088)
D2	AF354707
D3	L28111
Actin	AF079161

- For *in vivo* exposure to methimazole, perchlorate, and PTU, a concentration known to delay development was chosen and administered in a flow-through system. Treatment began with NF stage 54 tadpoles.
- Explant cultures of thyroid glands were acclimated for 24 hours before administration of TSH or co-cultured pituitary-conditioned media, as indicated.
- Data represent the mean ± SD (or SE in some plots) of 4 to 5 individuals.
- T_4 measurements by ELISA, RIA, and/or ICP-MS

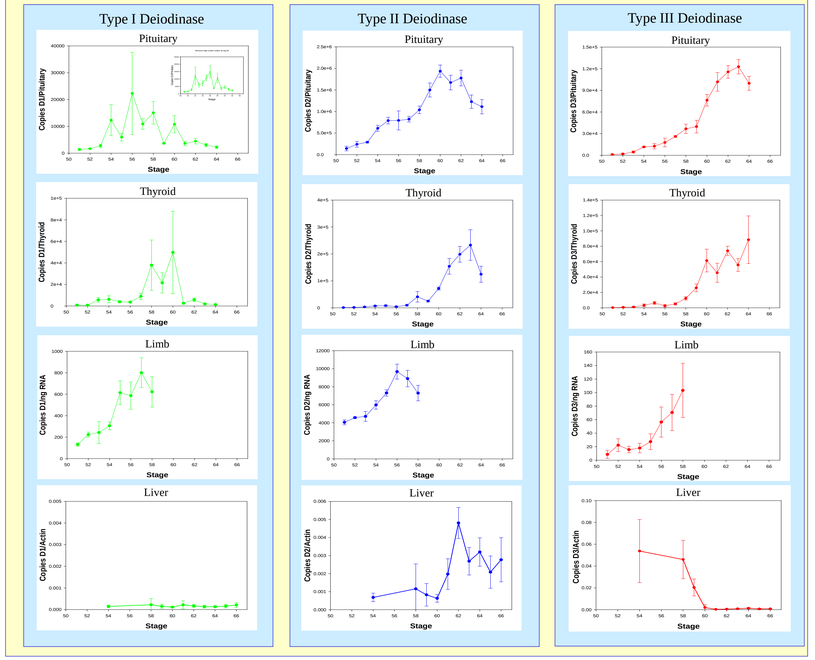
In vitro Thyroid Gland Studies



A preliminary ICP-MS analysis from thyroid glands cultured in the presence of bovine TSH or *X. laevis* pituitary conditioned media, showed measurable MIT and DIT and abundant T4 released, but no T3!

In vivo Exposures

Developmental Expression



Conclusions

- Deiodinase expression patterns show tissue and stage-specific changes consistent with roles determining the availability of T_3 and T_4.
- D1 expression, although often very low, also seems to be regulated in a tissue and stage specific manner. Recent evidence shows that in contrast to other D1s, *X. laevis* D1 is not inhibited by PTU, which may explain why this activity has not been reported.
- Deiodinase expression in liver does not support a role for this tissue in providing circulating T_3 before stage 60.
- Deiodinase expression in liver also suggests that another system, e.g. UDPGT pathway, may be responsible for TH elimination after stage 60.
- Deiodinase expression in the thyroid suggests a role in providing circulating T_3, but *in vitro* TH measurements show only T_4 release, not T_3.

References

Kuiper, G.G., et al. (2005) *Thyroid* 15, 787. Bianco AC., et al. (2002) *Endocrine Reviews* 23, 38. Brown, D.D. (2005) *Thyroid* 15, 815.